abcam

Product datasheet

Human VASP knockout HeLa cell line ab265892

画像数 3

製品の概要

製品名	Human VASP knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 13 bp deletion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
アプリケーション	**適用あり:** WB
Biosafety level	2
特記事項	**Recommended control:** Human wild-type HeLa cell line (**ab255928**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^4$ cells/cm^2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm^2 is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

Cells should be passaged when they have achieved 80-90% confluence.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

製品の特性

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Cervix
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female
STR Analysis	Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10
Antibiotic resistance	Puromycin 1.00µg/ml
Mycoplasma free	Yes
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

機能	Ena/VASP proteins are actin-associated proteins involved in a range of processes dependent on cytoskeleton remodeling and cell polarity such as axon guidance, lamellipodial and filopodial dynamics, platelet activation and cell migration. VASP promotes actin filament elongation. It protects the barbed end of growing actin filaments against capping and increases the rate of actin polymerization in the presence of capping protein. VASP stimulates actin filament elongation by promoting the transfer of profilin-bound actin monomers onto the barbed end of growing actin filaments. Plays a role in actin-based mobility of Listeria monocytogenes in host cells. Regulates actin dynamics in platelets and plays an important role in regulating platelet aggregation.
組織特異性	Highly expressed in platelets.
配列類似性	Belongs to the Ena/VASP family. Contains 1 WH1 domain.
ドメイン	The EVH2 domain is comprised of 3 regions. Block A is a thymosin-like domain required for G-actin binding. The KLKR motif within this block is essential for the G-actin binding and for actin polymerization. Block B is required for F-actin binding and subcellular location, and Block C for tetramerization. The WH1 domain mediates interaction with XIRP1.
翻訳後修飾	Major substrate for cAMP-dependent (PKA) and cGMP-dependent protein kinase (PKG) in platelets. The preferred site for PKA is Ser-157, the preferred site for PKG, Ser-239. In ADP-activated platelets, phosphorylation by PKA or PKG on Ser-157 leads to fibrinogen receptor inhibition. Phosphorylation on Thr-278 requires prior phosphorylation on Ser-157 and Ser-239. In response to phorbol ester (PMA) stimulation, phosphorylated by PKC/PRKCA. In response to

thrombin, phosphorylated by both PKC and ROCK1. Phosphorylation at Thr-278 by AMPK does not require prior phosphorylation at Ser-157 or Ser-239. Phosphorylation modulates F-actin binding, actin filament elongation and platelet activation. Carbon monoxide (CO) promotes phosphorylation at Ser-157, while nitric oxide (NO) promotes phosphorylation at Ser-157, but also at Ser-239. Response to NO and CO is blunted in platelets from diabetic patients, and VASP is not phosphorylated efficiently at Ser-157 and Ser-239.

細胞内局在

Cytoplasm. Cytoplasm > cytoskeleton. Cell junction > focal adhesion. Cell projection > lamellipodium membrane. Cell projection > filopodium membrane. Targeted to stress fibers and focal adhesions through interaction with a number of proteins including MRL family members. Localizes to the plasma membrane in protruding lamellipodia and filopodial tips. Stimulation by thrombin or PMA, also translocates VASP to focal adhesions. Localized along the sides of actin filaments throughout the peripheral cytoplasm under basal conditions.

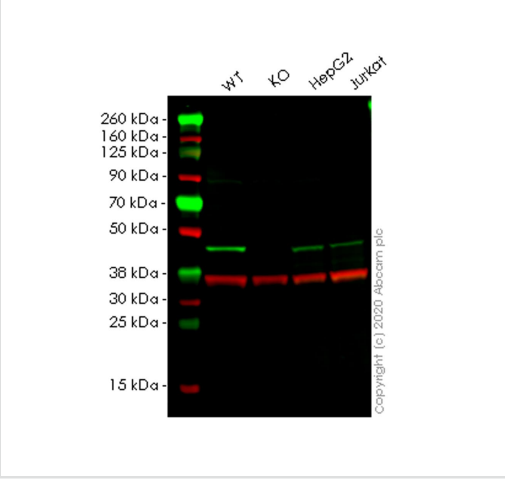
アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab265892の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 39 kDa.

画像



Western blot - Human VASP knockout HeLa cell line (ab265892)

All lanes : Anti-VASP antibody [EPR1337(2)] (**ab109321**) at 1/1000 dilution

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : VASP knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : HepG2 (Human liver hepatocellular carcinoma cell line) whole cell lysate
Lane 4 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

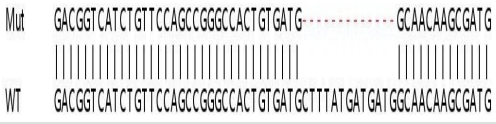
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 39 kDa
Observed band size: 46 kDa

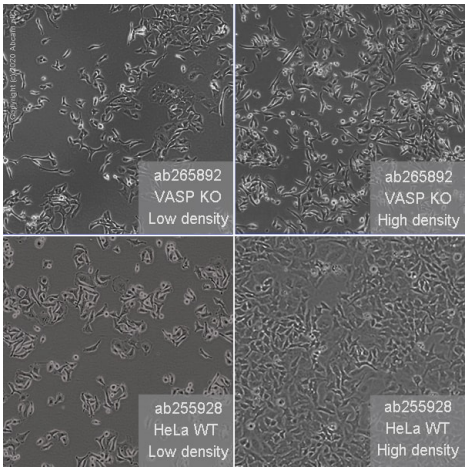
Lanes 1-4: Merged signal (red and green). Green - **ab109321** observed at 46 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab109321 Anti-VASP antibody [EPR1337(2)] was shown to specifically react with VASP in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265892 (knockout cell lysate **ab257792**) was used. Wild-type and VASP knockout samples were subjected to SDS-PAGE. **ab109321** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Sanger Sequencing - Human VASP knockout HeLa cell line (ab265892)

Homozygous: 13 bp deletion in exon 2.



Cell Culture - Human VASP knockout HeLa cell line (ab265892)

Representative images of VASP knockout HeLa cells, low and high confluency examples (top left and right respectively) and wild-type HeLa cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using a EVOS M5000 microscope.

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